

# ***Molecular Indicators of Genetic Diversity***

***Utility at local and regional scales***

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***What?  
Why?  
How?  
Examples***

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***What?***

*Why?*

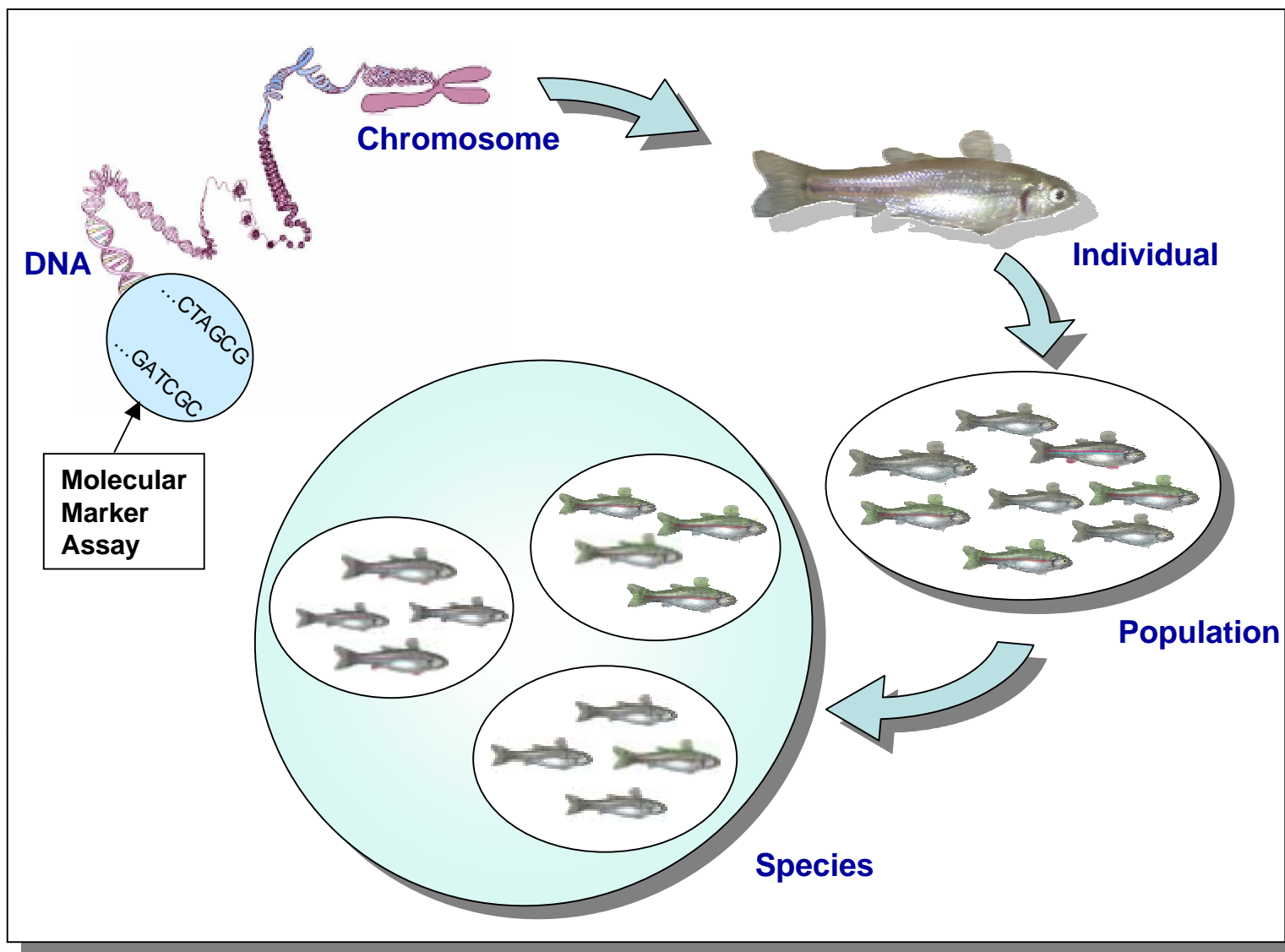
*How?*

*Examples*

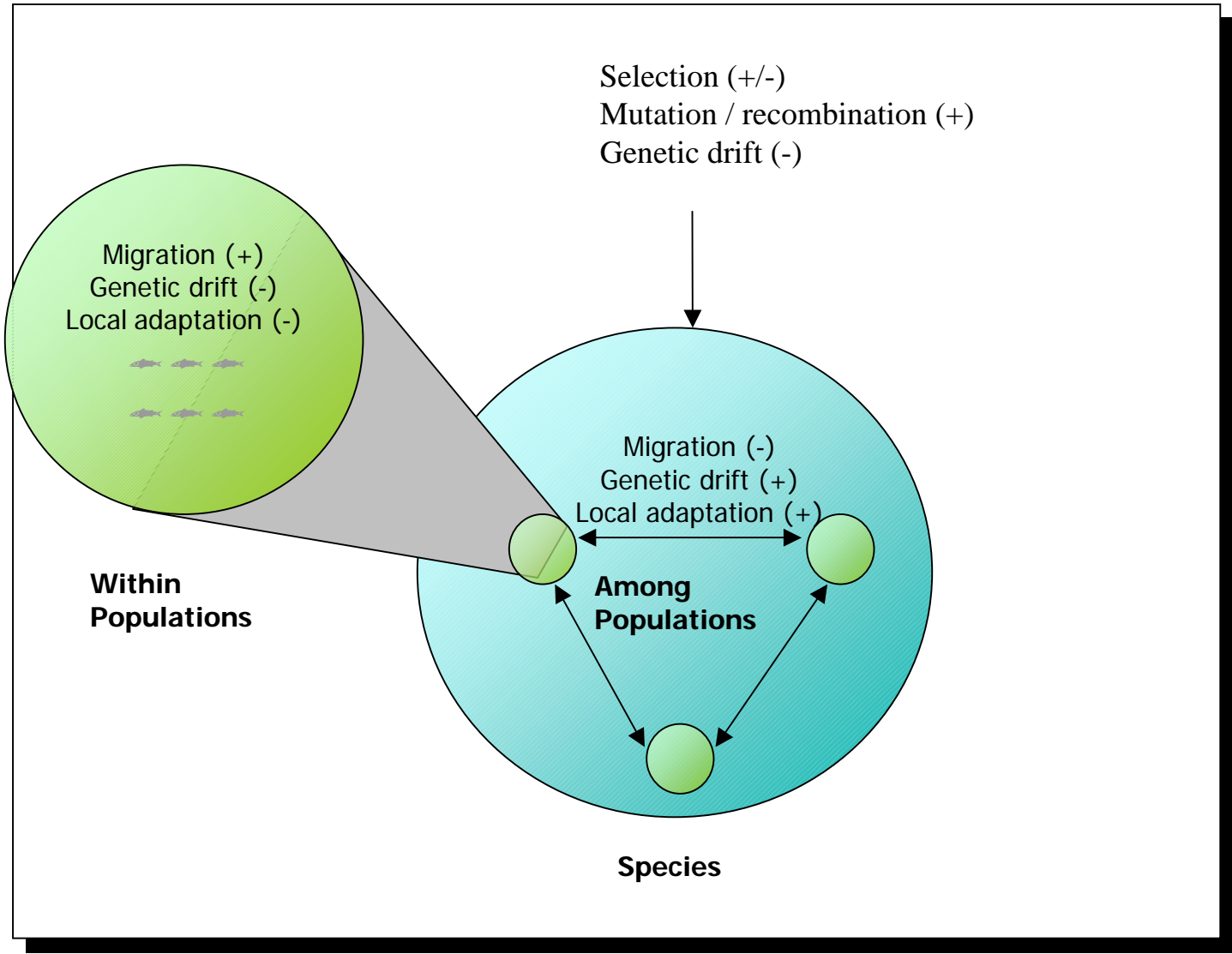
# *Genetic Diversity*

- Variation in the heritable differences of measurable traits that exists among individuals within a species.
  - Genetic diversity within populations
  - Genetic diversity among populations
- Examples: eye color, height, blood type, HIV resistance

# Molecular Population Genetic Approach



# Forces that act on genetic diversity



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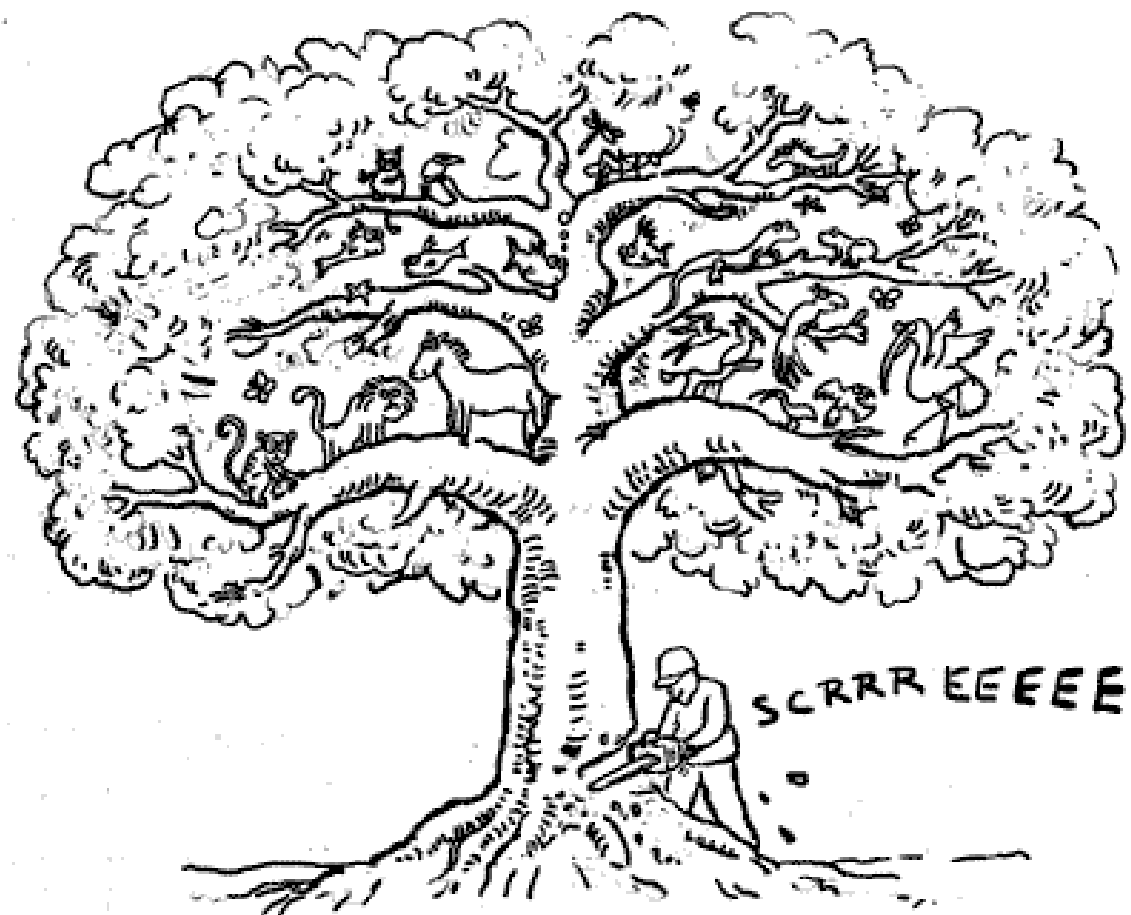
*What?*

***Why?***

*How?*

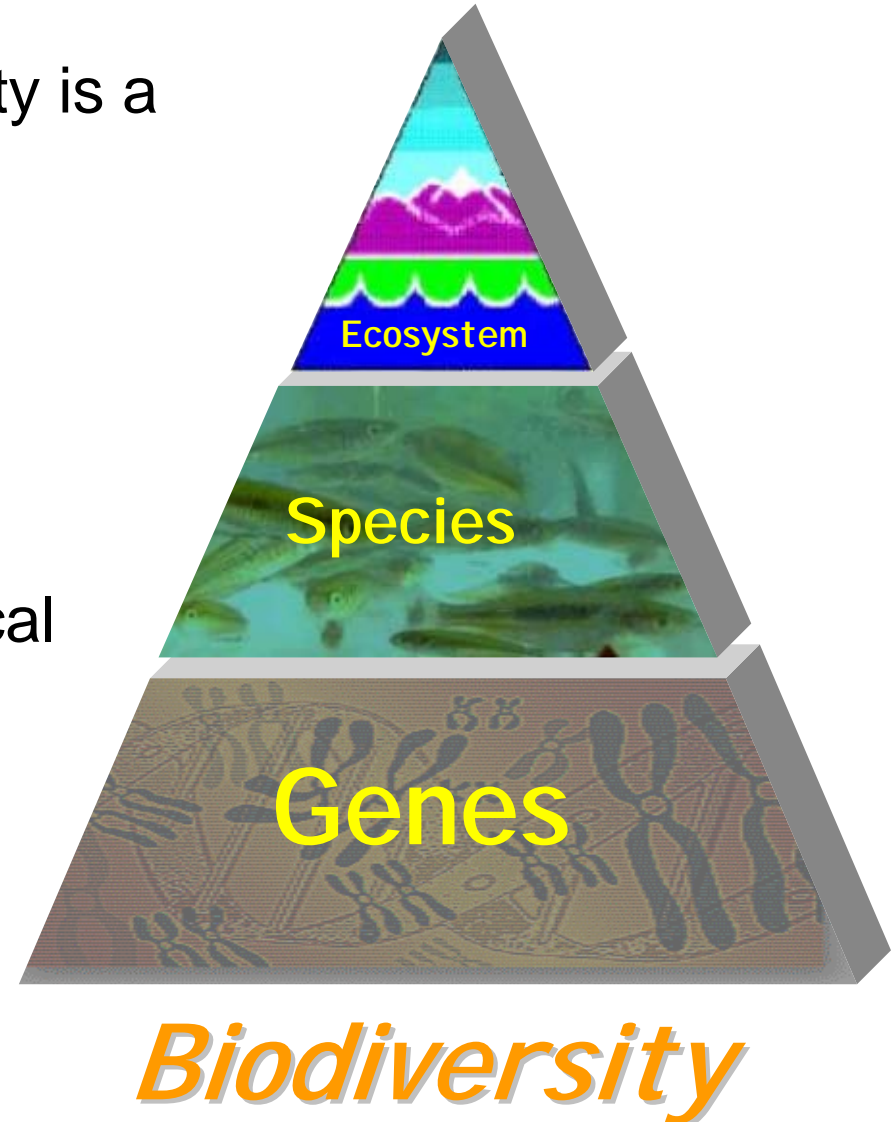
*Examples*

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THE TREE OF LIFE

- Genetic diversity is a fundamental component of biodiversity
- It defines a fundamental unit of ecological assessment

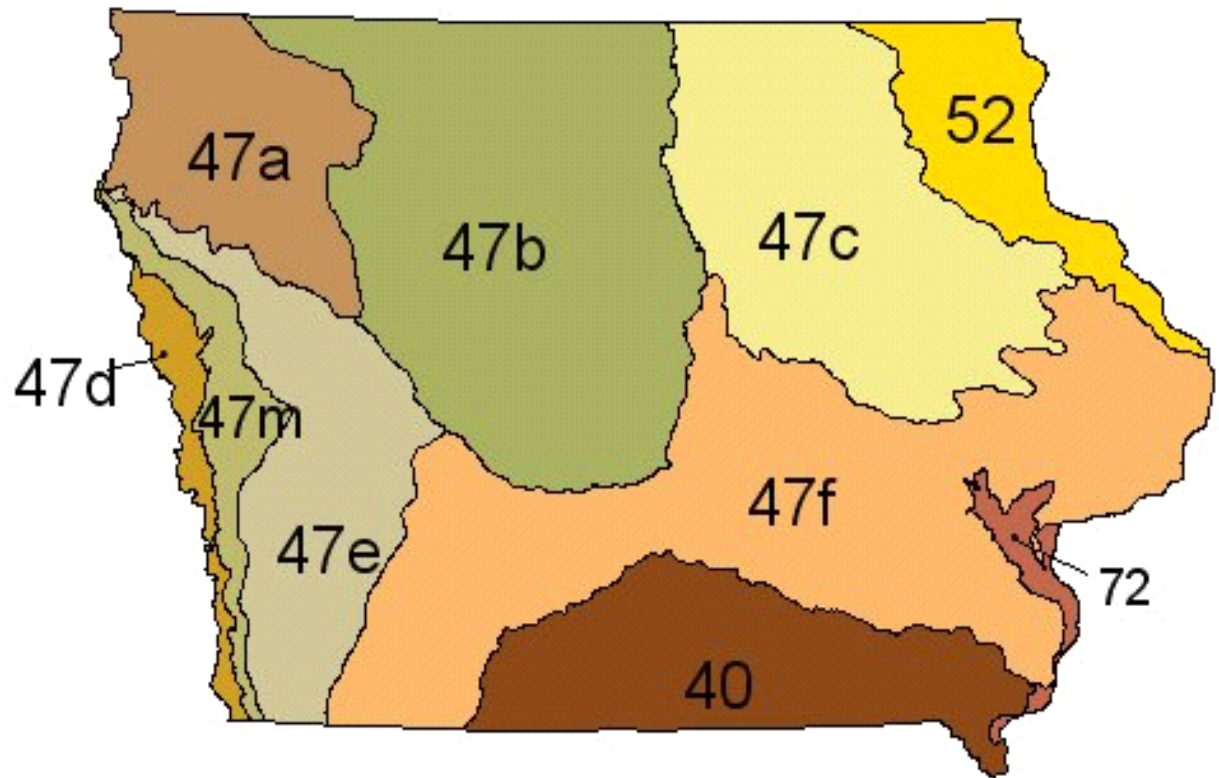


# What is the appropriate scale of assessment?



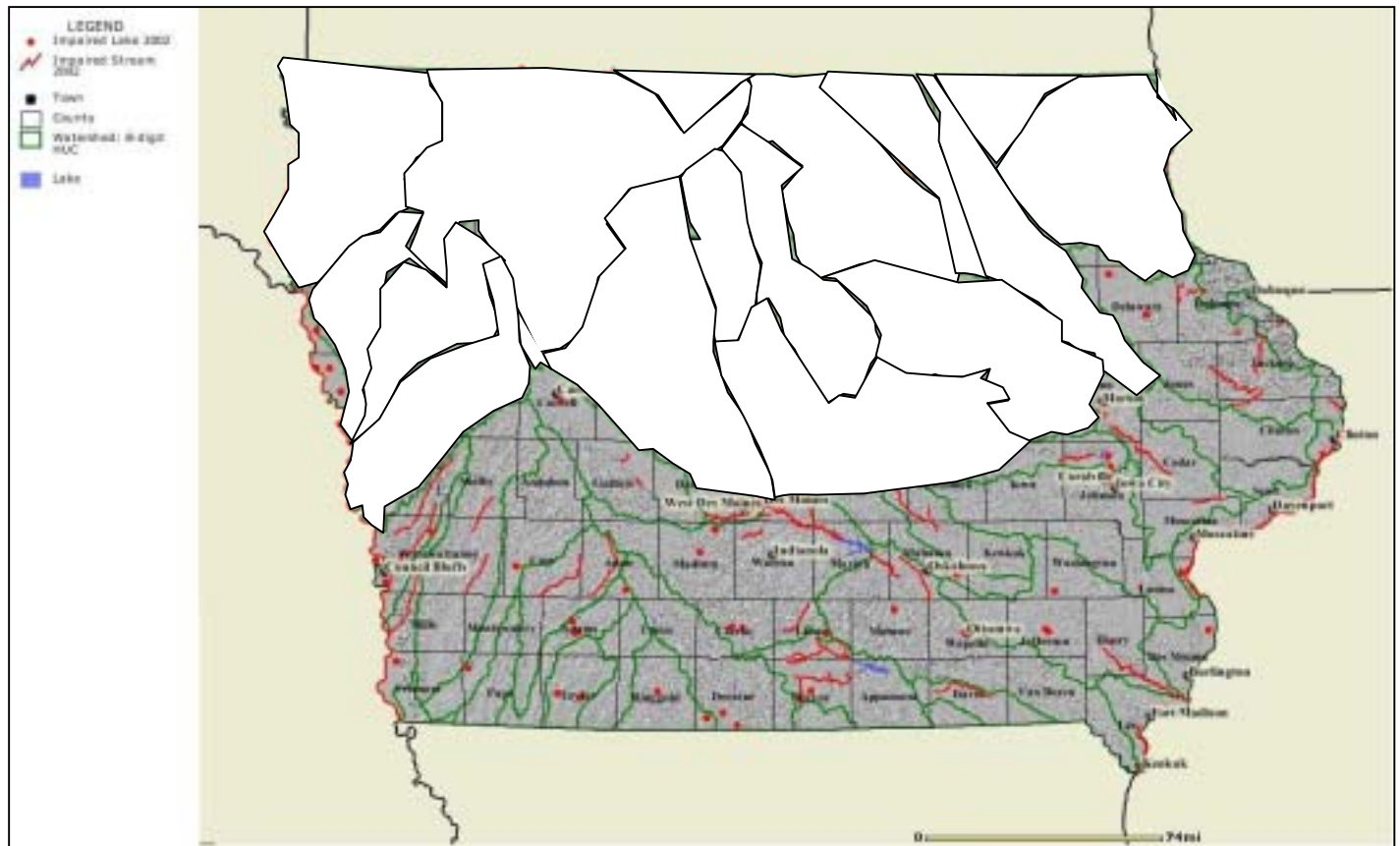
**Watershed**

## ***What is the appropriate scale of assessment?***



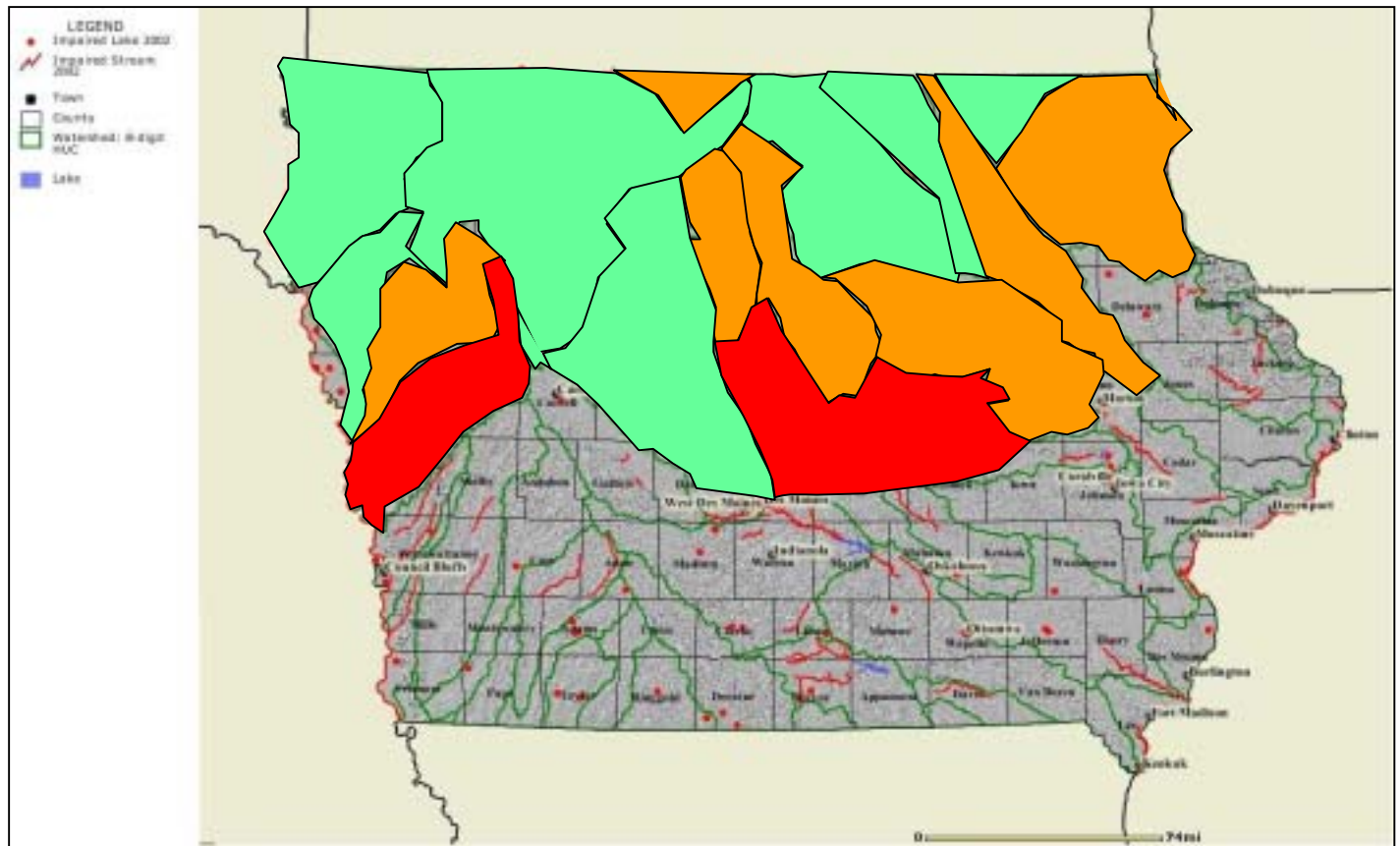
***Ecoregion***

# What is the appropriate scale of assessment?



**Resource Population**

# *What is the appropriate scale of assessment?*



**Resource Population**

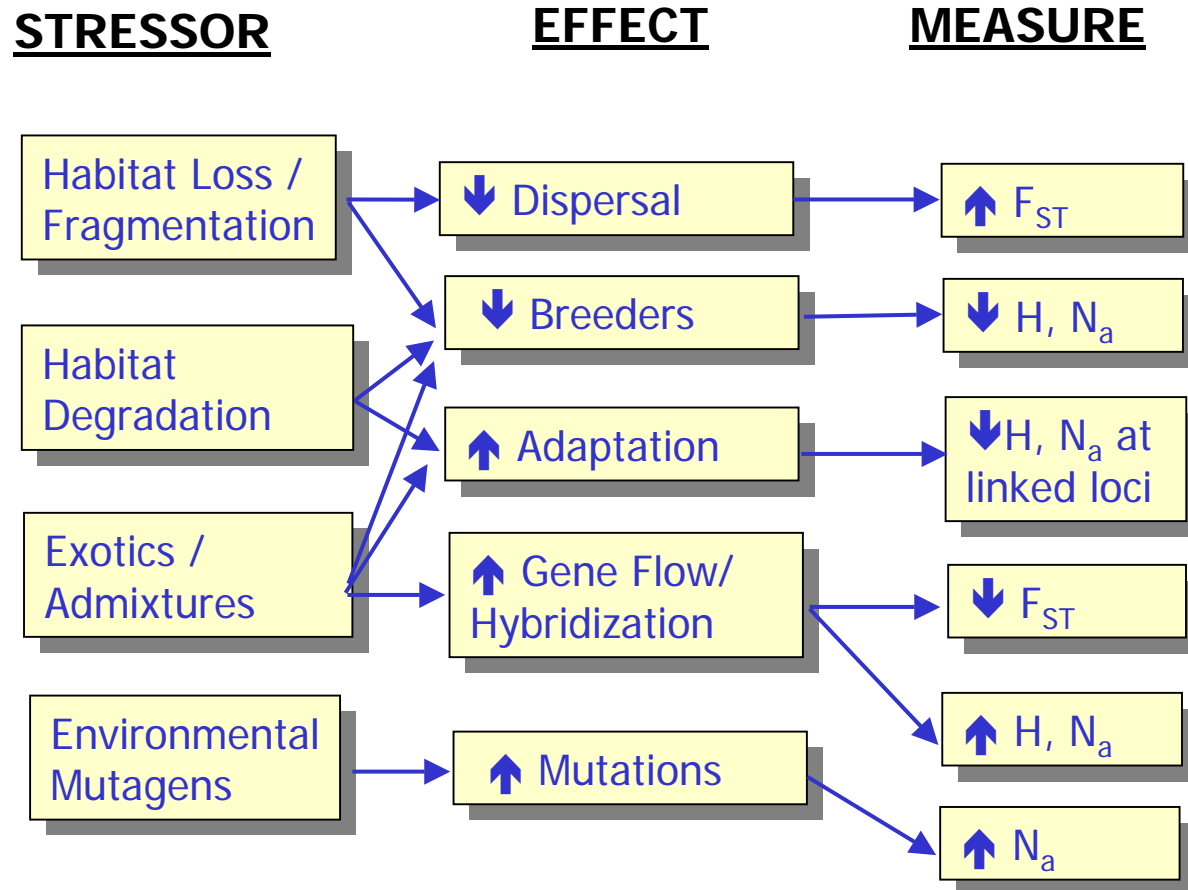
# Correlations between Genetic Diversity and Contaminant Exposure

Known Stressors	Molecular Marker	Taxa	References
<b>Mercury, other metals</b>	Allozyme	Shrimp, Fish, Insects, Amphipods, Molluscs	Nevo <i>et al.</i> , 1984, Benton <i>et al.</i> , 1994, Heagler <i>et al.</i> , 1993, Keklak <i>et al.</i> , 1994, Roark and Brown, 1996, Diamond <i>et al.</i> , 1989, Mulvey <i>et al.</i> , 1995, Tatara <i>et al.</i> , 1999, Ben-Shlomo and Nevo, 1988, Lavie and Nevo, 1982, 1986b, Benton and Guttman, 1992a,b, Chagnon and Guttman, 1989, Schlueter <i>et al.</i> , 1995, 1997, 2000, Duan <i>et al.</i> , 2000a, Moraga <i>et al.</i> , 2002, Larno <i>et al.</i> , 2001
<b>Acidity, [Al]</b>	Allozyme	Fish, Amphipod	Kopp <i>et al.</i> , 1992, Duan <i>et al.</i> , 2000a
<b>Arsenate</b>	Allozyme	Fish	Newman <i>et al.</i> , 1989
<b>Pesticides</b>	Allozyme	Fish, Bivalve	Hughes <i>et al.</i> , 1991, Brown Sullivan and Lydy, 1999, Tanguy <i>et al.</i> , 1999
<b>PAH (fluoranthene)</b>	Allozyme	Fish, Amphipod	Schlueter, <i>et al.</i> , 2000, Duan <i>et al.</i> , 2000b, Larno <i>et al.</i> , 2001
<b>Radionuclides</b>	Allozyme, RAPD	Fish	Theodorakis and Shugart, 1997, 1998
<b>Overall water quality, complex effluents</b>	Allozyme, CYP1A, Sequence, MtDNA, RAPD	Fish, Copepod, Crayfish, Mussel, Barnacle	Gillespie and Guttman, 1989, 1993, Foré <i>et al.</i> , 1995a,b, Heithaus and Laushman, 1997, Roy <i>et al.</i> , 1996, Murdoch and Hebert, 1994, Street and Montagna, 1996, Nadig <i>et al.</i> , 1998, Krane <i>et al.</i> , 1999, Ma <i>et al.</i> , 2000, Roark <i>et al.</i> , 2001.

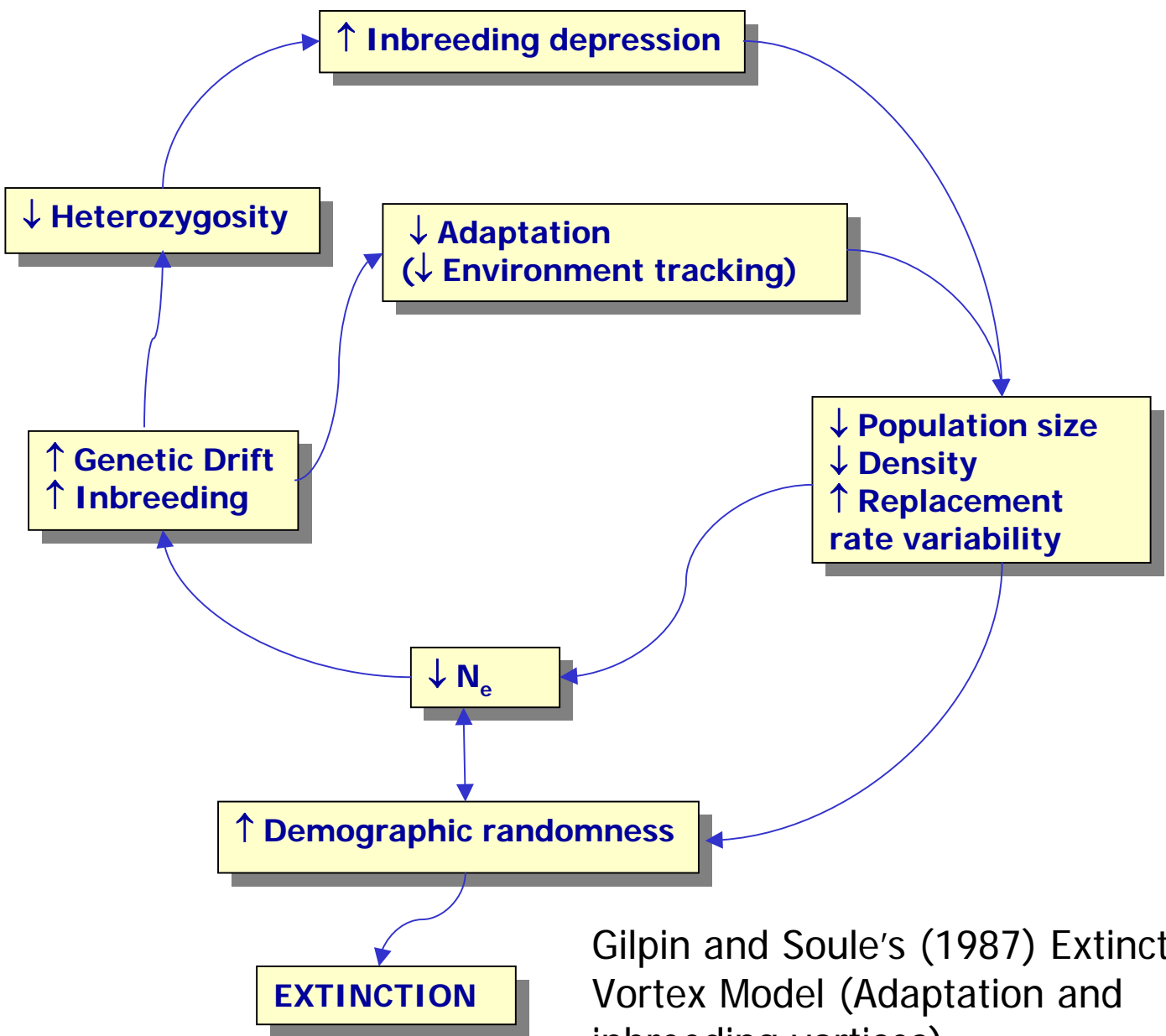
## *Correlations between Genetic Diversity and Fitness*

<b>Fitness Endpoint</b>	<b>Mol. marker</b>	<b>Taxon</b>	<b>Reference</b>
Local population extinction	Allozyme, microsatellite	Butterfly	Saccheri et al., 1998
Lifetime breeding success	Microsatellite	Red deer	Slate et al., 2000
Colony growth and survival	Allozyme	Ant	Cole and Wiernsasz, 1999
Fertility, hatching rate	Microsatellite	Prarie chicken	Westemeir et al., 1998; Bouzat et al., 1998
Mortality, growth, fecundity, developmental stability	Allozyme	Topminnow	Quattro and Vrijenhoek, 1989
Developmental stability	mtDNA, microsatellites	Elephant Seal	Hoelzel et al., 2002
Male reproductive success	Allozyme	Butterflies (2 species)	Carter and Watt, 1988
Male reproductive success	Allozyme	Gastropod	Rolan-Alvarez et al., 1995
Birth wt., neonatal survival	Microsatellite	Seal	Coltman et al., 1998

# Predictable Stress-Response-Measure Relationship

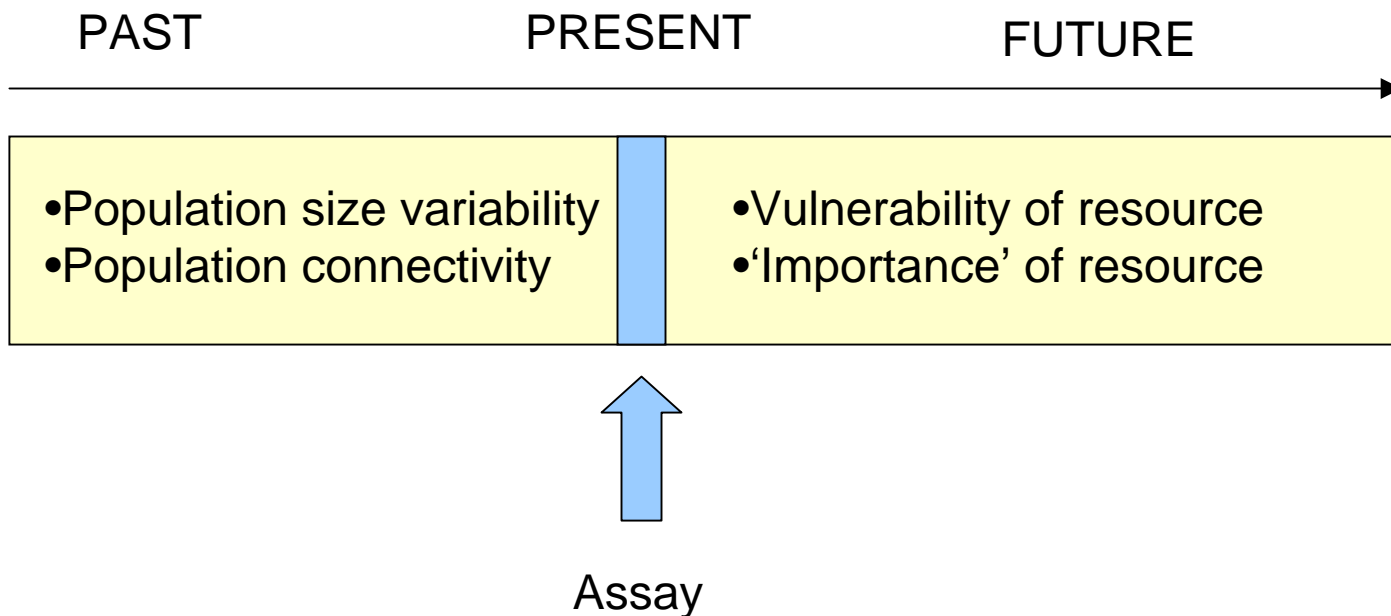


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Gilpin and Soule's (1987) Extinction Vortex Model (Adaptation and inbreeding vortices)

## ***Prospective and Retrospective Indicator***





National Wildlife

"Maybe we'd better leave this one alone."

## ***Why? - summary***

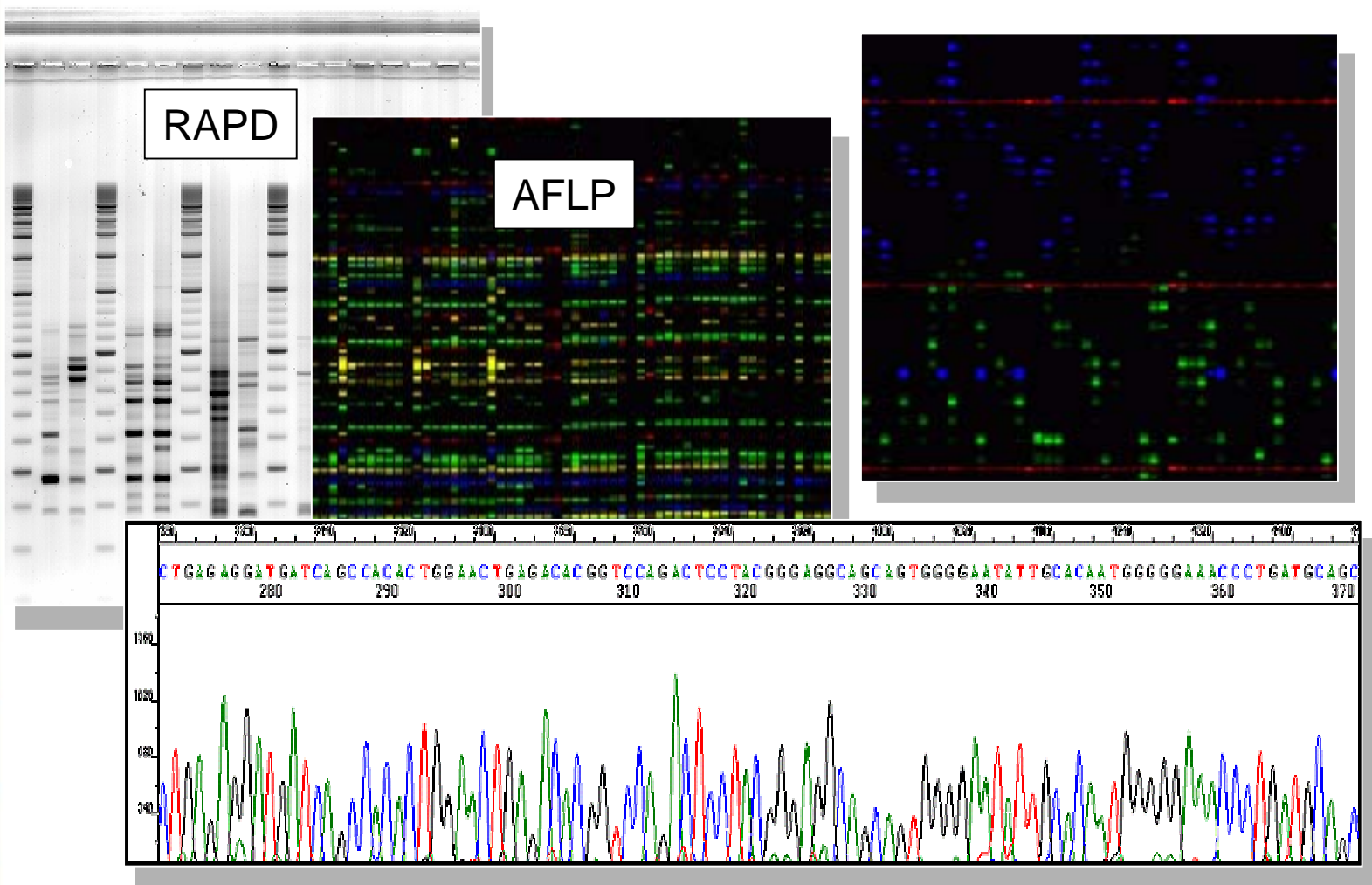
- Genetic diversity is a fundamental component of biodiversity
- Stressors affect genetic diversity in predictable ways (ecological indicator)
- Genetic diversity limits potential responses to future stressors (sustainability indicator)
- Understanding of genetic diversity patterns and population structure enhances the value and interpretation of other ecological assessment data

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*What?*  
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*Examples*

# DNA fingerprints

# Microsatellites

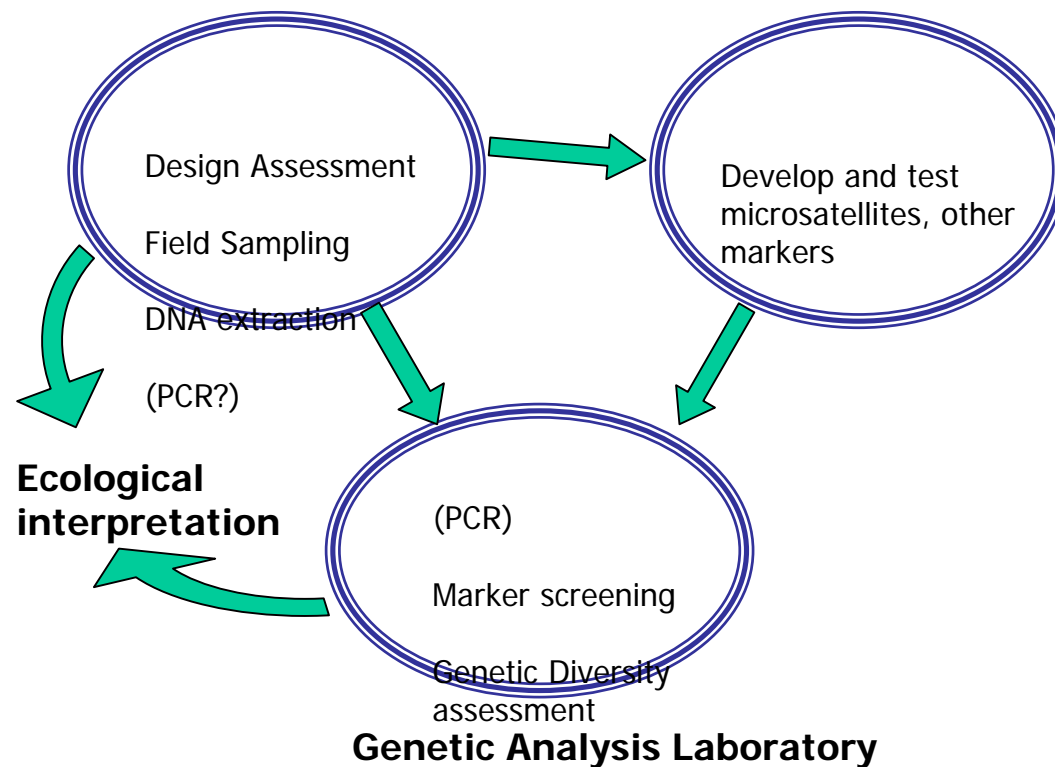


DNA Sequences

## *High technology may require coordination with specialized labs*

### Regional Laboratory

### Marker Development Laboratory



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*What?*

*Why?*

*How?*

*Examples*

## *Population genetics in the EPA*

- Biotechnology Risk- Resistance development in pests targeted by Bt toxins, effects on non-target insects
- Invasive Species- source-tracking, introgressive hybridization, and invasion dynamics
- Monitoring- Genetic taxonomy and enumeration of cryptic invertebrate samples in stream, lake and ballast samples
- Landscape Genetics- integration of landscape and genetic information into population models
- Ecological Assessment- Watershed and regional analysis of fish and invertebrate populations



## Examples of Ecological Assessments

I. Within watershed



II. Several watersheds



III. Regional assessment

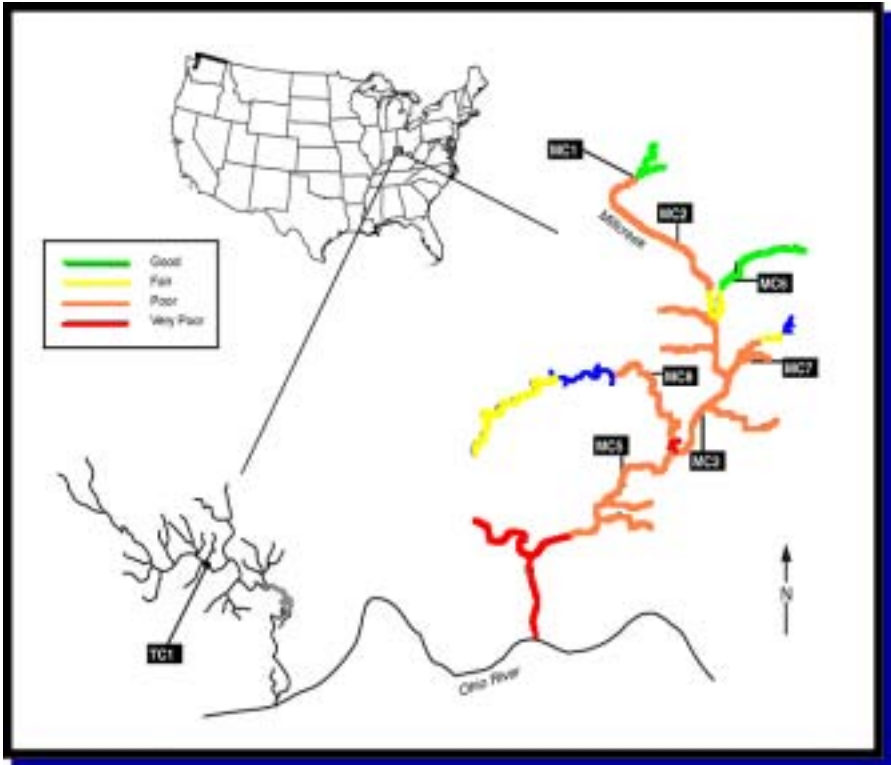
# ***I. Temporal analysis of population sizes and migration rates for a stream minnow in a small urban watershed***

## **Goals:**

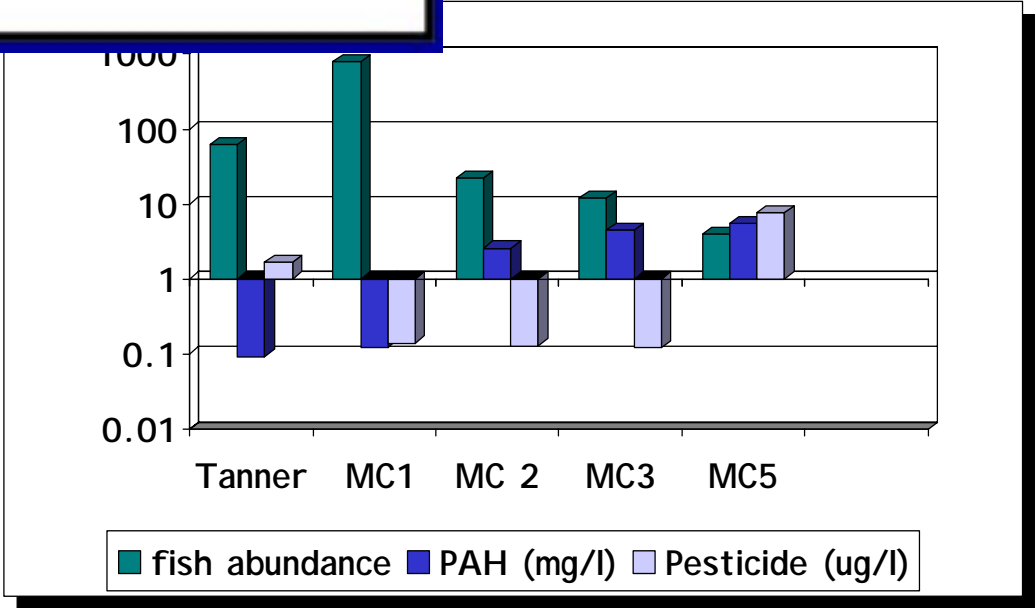
- Determine whether population genetic structure exists within a single watershed
- Determine whether local effective population sizes and immigration rates within a watershed are related to habitat quality



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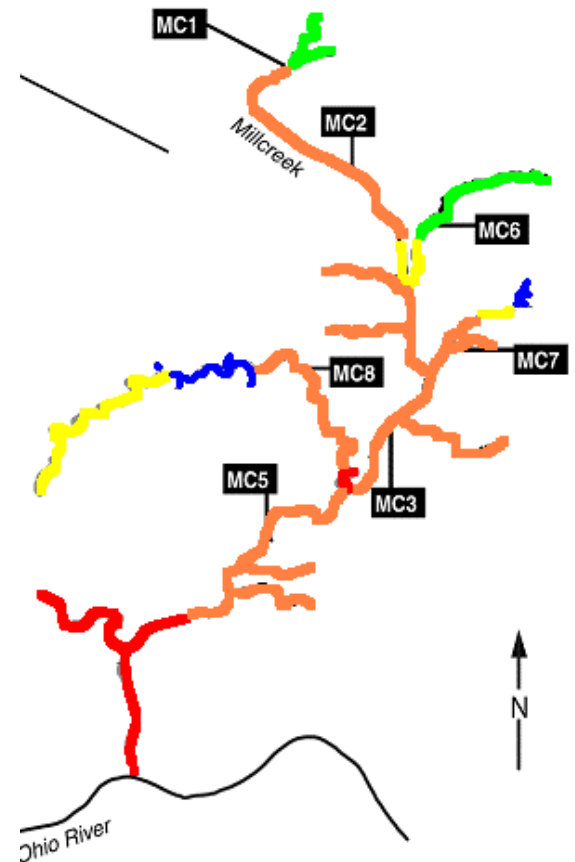


# Mill Creek Watershed, Cincinnati, OH



## Genetic Analysis

- 4 mainstem, 3 tributary, and one 'control' site sampled in 2001-2002
- 4 mainstem sites also sampled in 1994-1995
- All samples genotyped at 10 microsatellite loci



## ***Hierarchical analysis of genetic structure***

Variance component	d.f.	Proportion of genetic variance	P
watersheds	1	1.17%	0.12
sites within Mill Creek	6	0.59%	0.00
years within sites	5	0.13%	0.21
Within sites	782	98.23%	

## ***Estimates of local effective sizes and immigration rates***

Site	$N_e$ (95% CI)	$m$ (95% CI)
MC1	57 (42-85)	0.57 (0.43-0.74)
MC2	12 (7-20)	<b>1.00</b> (0.67-1.00)
MC3	44 (31-71)	0.59 (0.41-0.87)
MC5	86 (47-116)	<b>1.00</b> (0.69-1.00)

Source-sink dynamics?

## II. Genetics of Creek Chubs in a Mining-Impacted Region

### Goals:

- Assess the relationship between USGS hydrologic units and genetic structure for creek chubs.
- Assess relationship between genetic diversity and measures of ecological condition

*Semotilus atromaculatus*



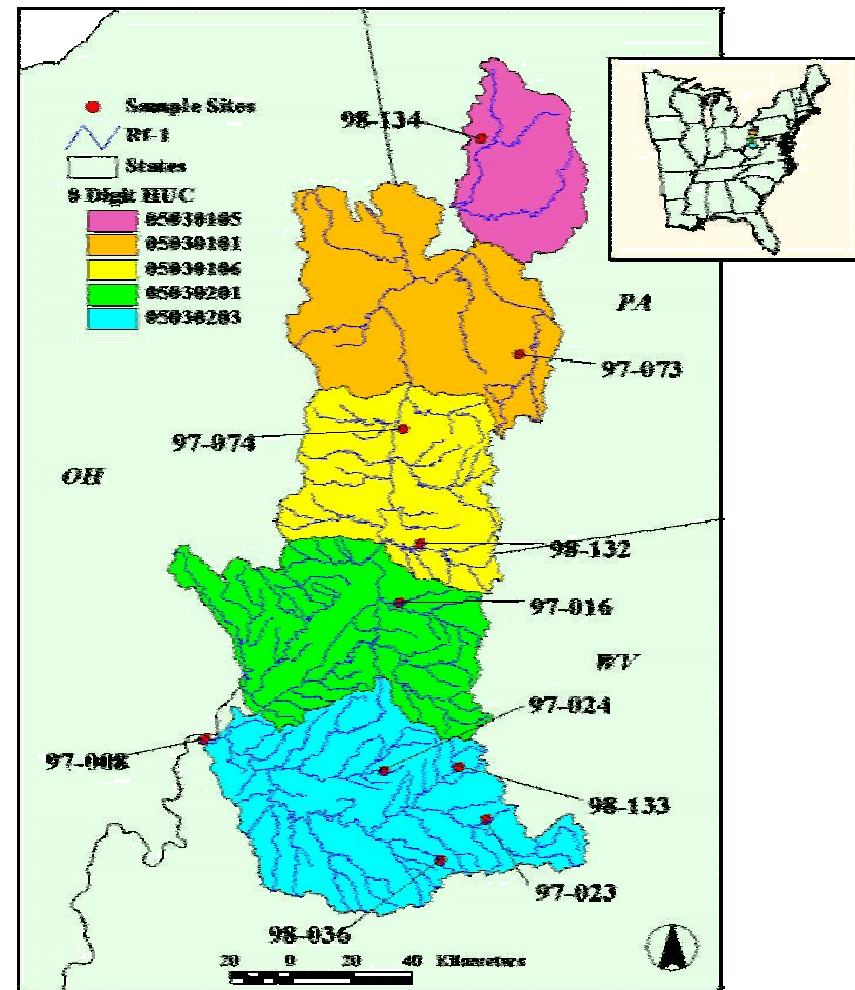
Photo courtesy of Ohio Dept. Natural Resources

## Study Sites

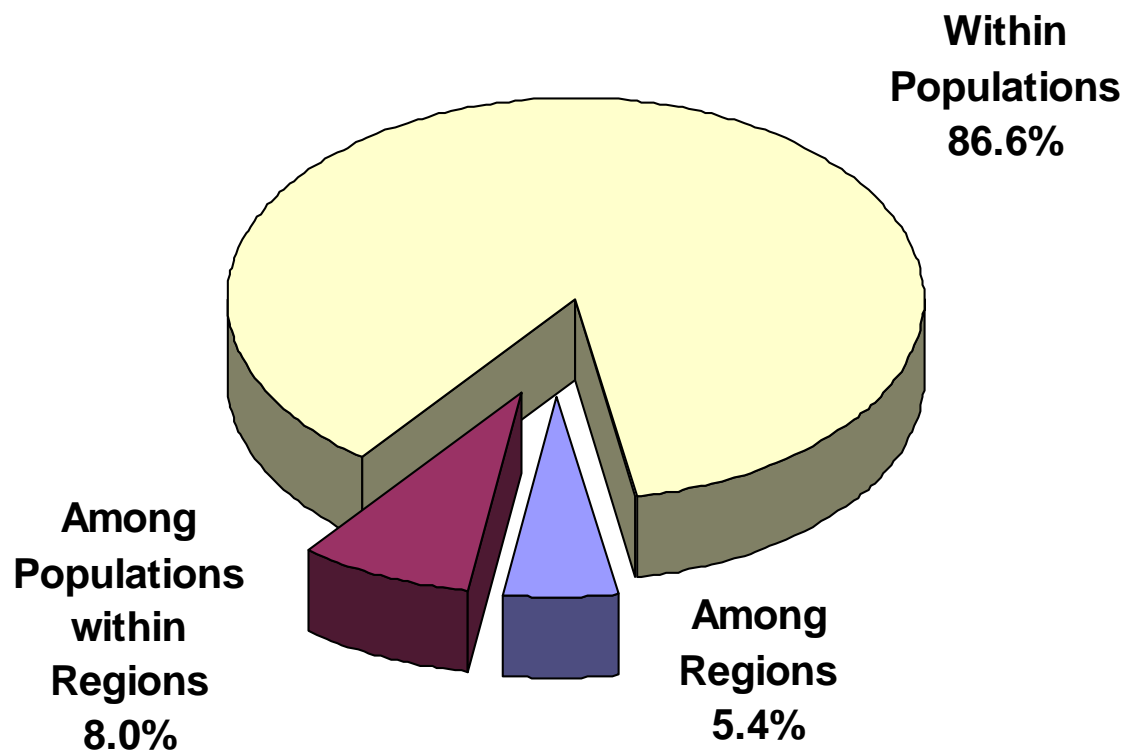
- 10 sample sites
- Part of EMAP-MAIA
- Agricultural-mining
- Wadeable streams

## Genetic Analysis

- 10-28 fish per site
- mtDNA sequences
- AFLP fingerprints
- Assess genetic differences within and among sites



## Genetic Structure – Nuclear DNA



## ***Principal Components Analysis to Classify Environmental Variation***

Principal component	Environmental variation explained	Variables
1 (Geochemistry)	37.4%	Conductivity, aluminum, calcium, chloride, potassium, magnesium, sodium, sulfate
2 (N/P/C)	24.3%	Nitrate, total nitrogen, total phosphorus, organic carbon
3 (Latitudinal clines)	14.1%	Latitude, elevation, channel slope, silica, zinc
4 (Spatial scale)	11.8%	Watershed area, stream width, stream depth
5 (pH-Ammonium)	6.7%	pH, Ammonium
6 (Substrate condition)	6.4%	Pebble size, embeddedness, percent riffle

## Stepwise multiple regression – nuclear DNA diversity

<i>PCA Factor</i>	<i>partial <math>R^2</math></i>	<i>model <math>R^2</math></i>	<i>F value</i>	<i>Pr &gt; F</i>
<i>PCA 3 (Latitudinal clines)</i>	0.4328	0.4328	6.10	0.0387
<i>PCA 2 (N/P/C)</i>	0.3489	0.7917	12.06	0.0104
<i>PCA 5 (pH/Ammonium)</i>	0.1841	<b>0.9758</b>	45.60	0.0005

98% of the differences in genetic diversity within populations explained by geographic and environmental factors

### ***III. Regional profile of fish genetic diversity in Eastern Cornbelt Plains Ecoregion***

#### **Goals:**

- Assess the relationship between HUCs and genetic structure for central stonerollers.
- Assess relationship between genetic diversity and ecological condition
- Ultimately, perform a multispecies assessment across much of the eastern USA

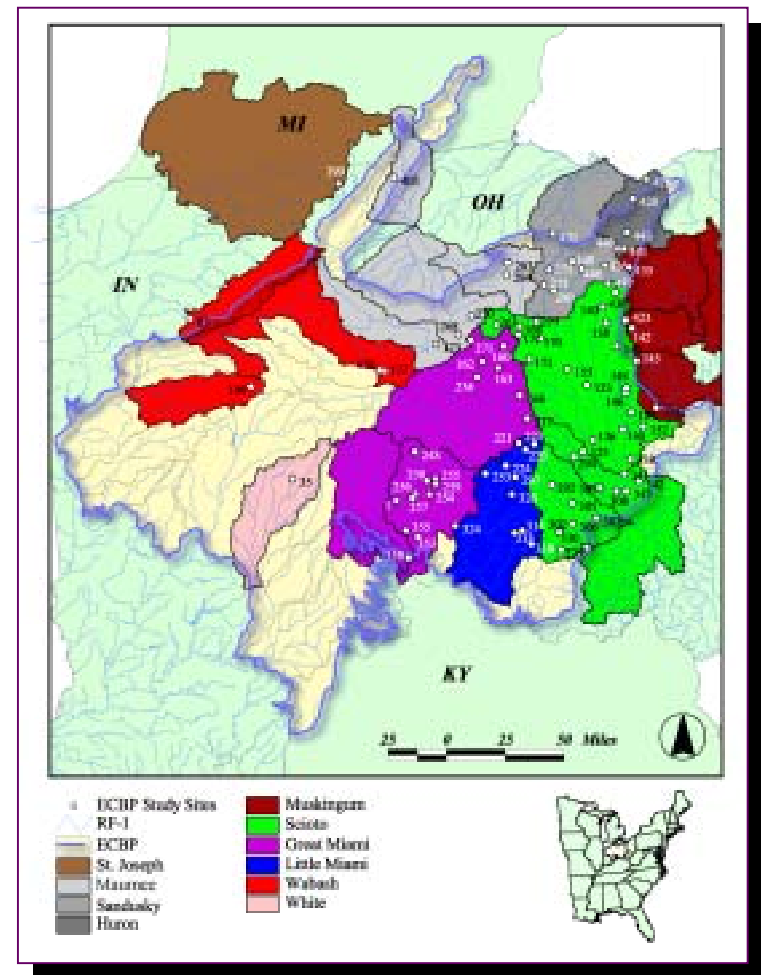


## Study sites

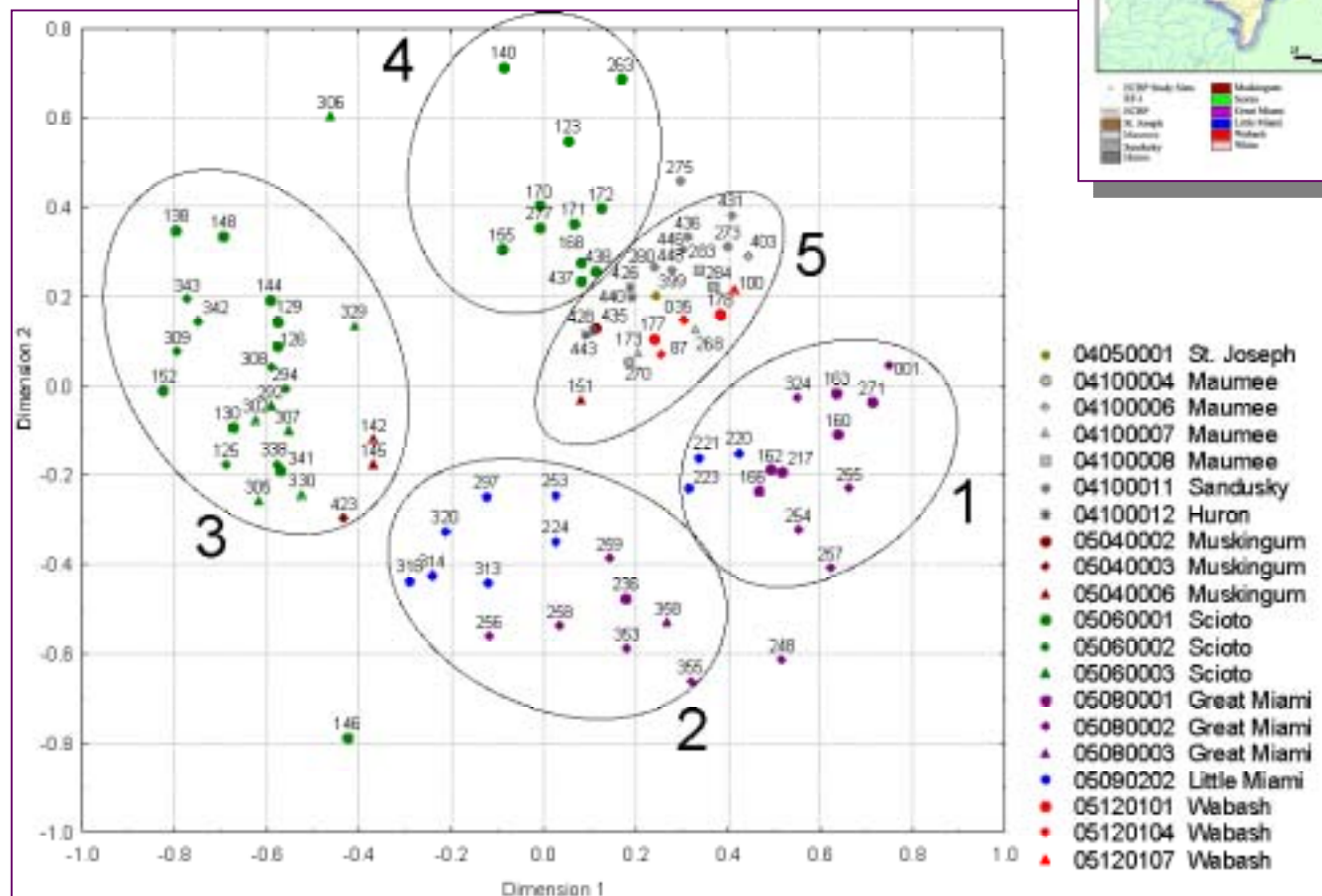
- 91 wadeable streams in ECBP
- Part of Regional EMAP study (probability-based sampling)
- Intensive ecological site characterization

## Genetic analysis

- RAPD fingerprints
- 3-10 stonerollers collected per site
- Assess genetic differences within and among sites

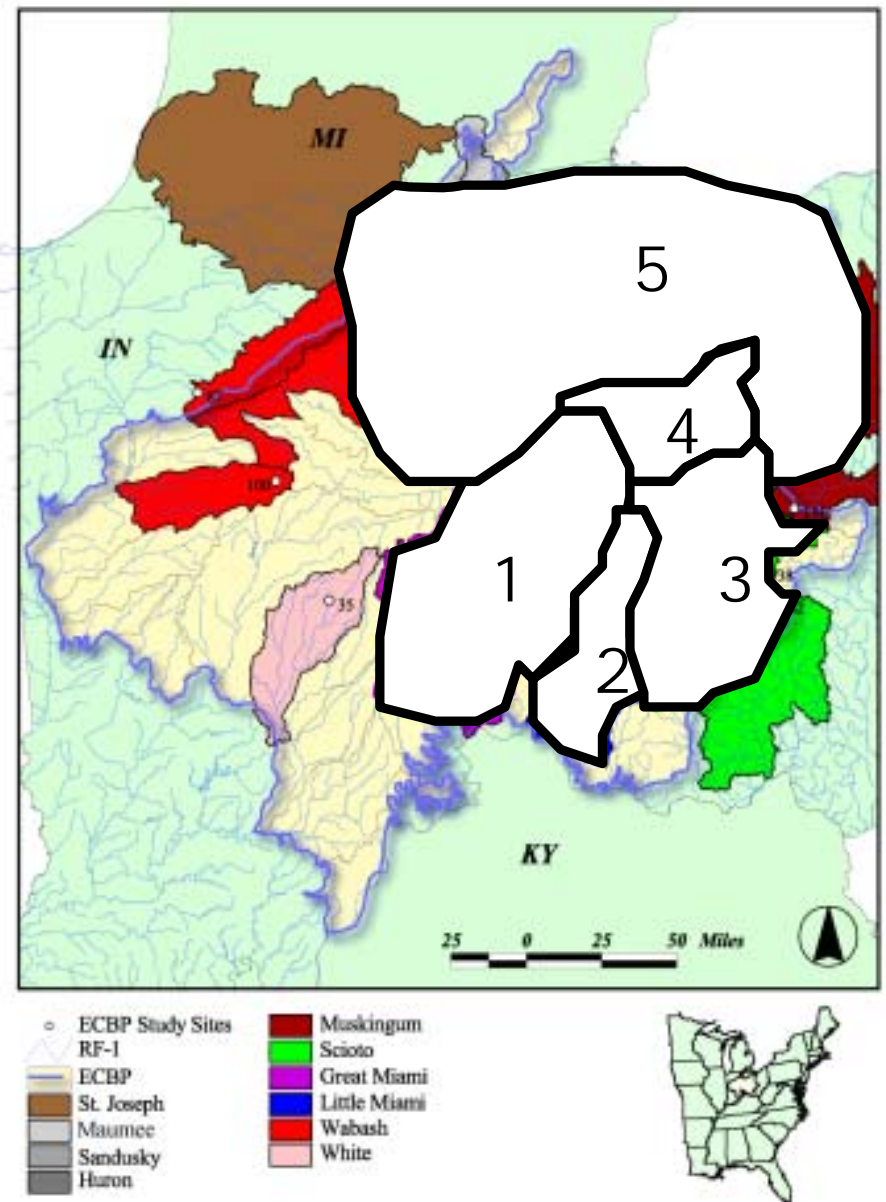


## Multidimensional Scaling



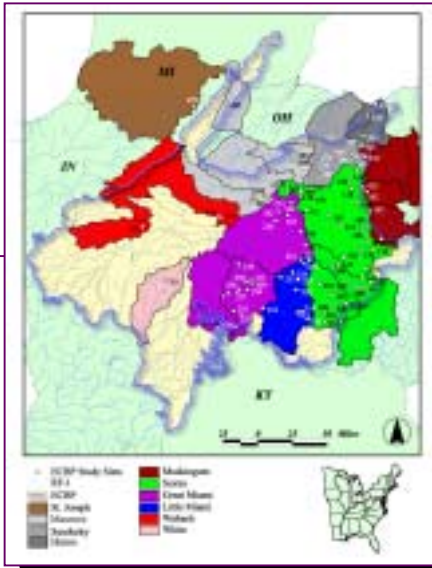
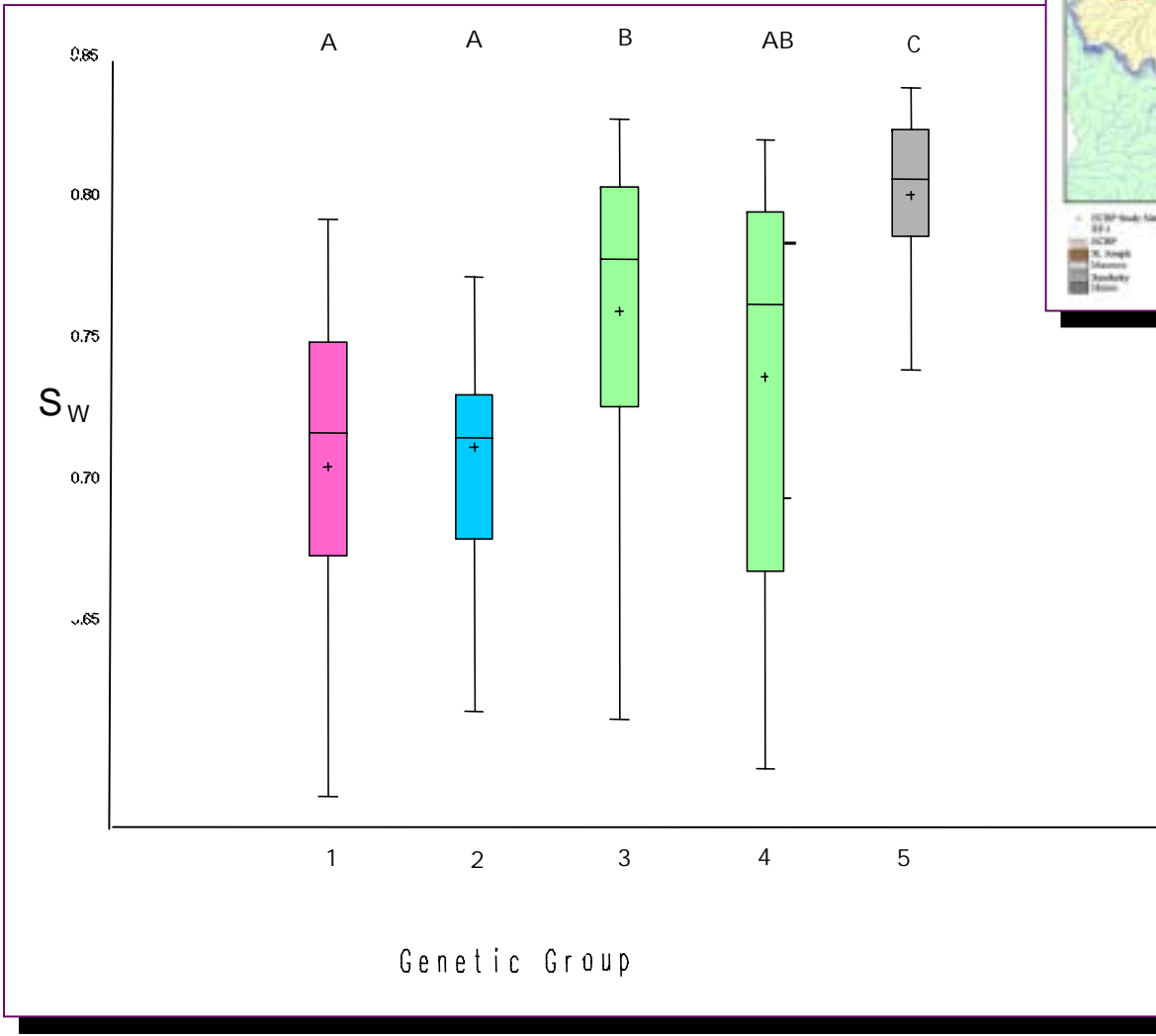
## 5 genetic groups

- More differentiation among southern watersheds
- Groupings related to watershed boundaries- but not exactly
- Fundamental units for ecological analysis?



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# Genetic Diversity within Genetic Groups



# **Multivariate analysis of differences in genetic diversity**

## **“Exposure” variables**

- Qualitative Habitat Evaluation Index
- BAP ( $\mu\text{g}/\text{mg}$  protein)
- NAPH ( $\mu\text{g}/\text{mg}$  protein)
- EROD ( $\text{pmol}/\text{min}/\text{mg}$  protein)
- Impacted- Urban
- Impacted –Agriculture
- Impacted- Riparian
- Impacted –Channelization

## **“Effects” variables**

- No. Fish (per 300 m)
- Wt. Fish ( $\text{kg}/300\text{ m}$ )
- No. Fish species
- Shannon Diversity Index
- Index of Well Being
- Index of Biotic Integrity

## **Geography and Scale variables**

- Ave. Sampling Depth
- Latitude
- Longitude
- Stream Order (1 – 3)
- Watershed Area
- Elevation
- Major Genetic Group

## ***Final model following stepwise elimination of least significant effects***

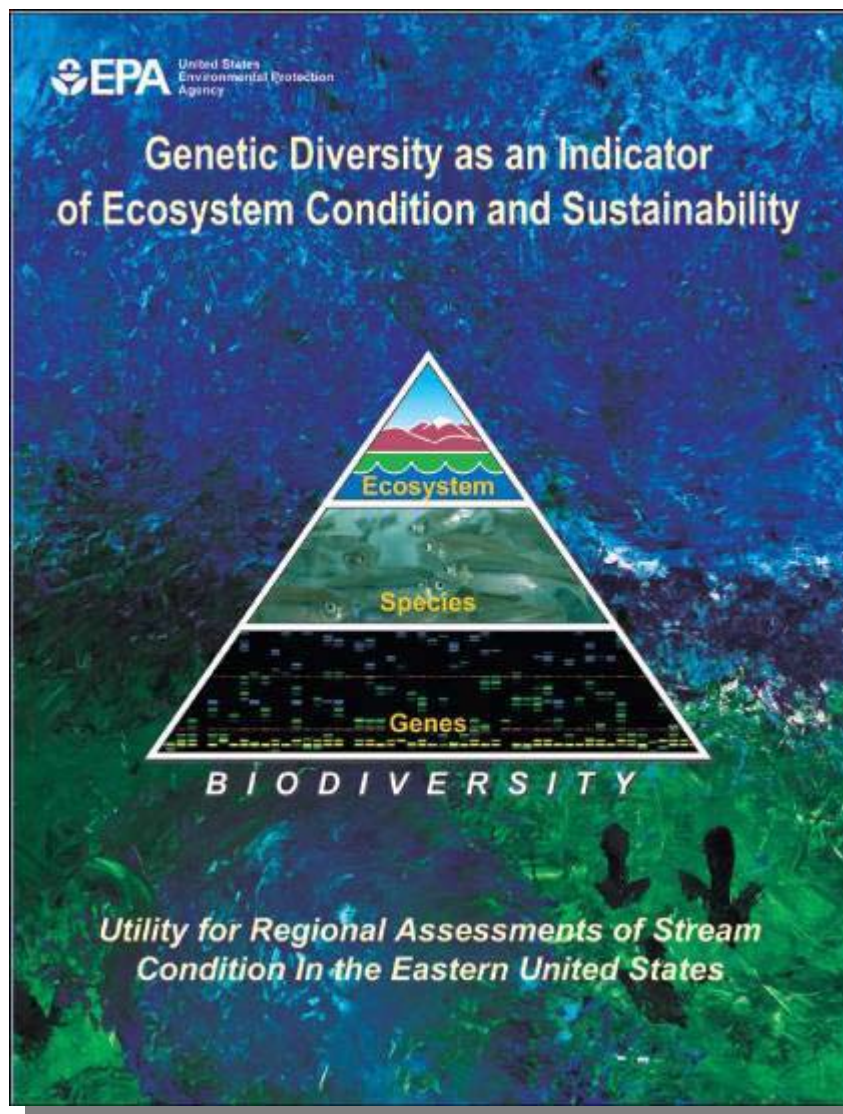
Source	D.F	Mean Square	F	Prob.
Impact factor – Urban	1	5.369	11.2	0.001
Impact factor – Riparian	1	4.882	10.2	0.002
Impact factor – Channelization	1	2.452	5.1	0.027
Major Genetic Group	4	6.311	13.1	< 0.001
Depth (covariate)	1	1.974	4.1	0.046

 **$R^2=0.53$**

## **Take-home Messages**

- Understanding population structure is key to understanding risks to biological resources
- Population genetic methodologies are effective tools for evaluating population structure at various scales and may now begin to tell us about source-sink dynamics
- Population-level responses to environmental change are reflected in genetic measurements
- Genetic diversity affects population vulnerability. The strength of this relationship still needs much more research.

***For detailed information see:***



## **Acknowledgements**

- US EPA: Suzanne Christ, Susan Franson, Eric Waits, Tom Wessendarp, Annette Roth, Tammy Goyke, Greg Toth
- Ohio EPA
- Sobran: Tony Leonard, Manju Garg, Ana Braam, Jared Smith, Richard Converse, Paul Weaver